AD-A167 267 ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES(U) NEW MEXICO UNIV ALBUQUERQUE SCHOOL OF MEDICINE R G DEAN 30 NOV 85 N00014-82-K-0680 1/1

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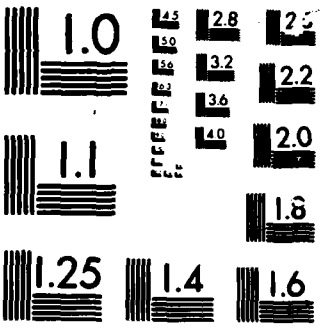

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NR 202-259

DEFENSE ADVANCED RESEARCH PROJECTS AGENCY

R & D STATUS REPORT

PROGRAM CODE NO.
#N00019

CONTRACT NO.
N00014-82-K-0680

SHORT TITLE OF WORK

ULTRASENSITIVE DETECTION OF CHEMICAL SUBSTANCES

REPORTING PERIOD
SEPTEMBER 1, 1985 -- NOVEMBER 30,1985

EFFECTIVE DATE OF CONTRACT
DECEMBER 1, 1984

EXPIRATION DATE OF CONTRACT
NOVEMBER 30, 1985

QUARTERLY REPORT

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In the past quarter we have concentrated our effort on completing task that have been initiated earlier in the year. In particular, we have focused on our Rivanol studies. For some time we have worked to enhance binding of trapping agents, proteins, to surfaces and at the same time reduce non-specific binding or noise. Another concern has been to bind trapping agents so that they would remain in an active configuration. Our discovery that Rivanol, an agent used in the precipitation of antibodies, enhances protein binding to polystyrene lead us to investigate its possible use as a binding agent. Our study of Rivanol has had many stumbling blocks, however after an intensive study we are now taking steps to apply for a patent covering the use of Rivanol as a binding agent. Below we This report covers the salient points of our work with Rivanol. Some of these results appear in previous reports. Cont'd

A stock solution of 15mM Rivanol in distilled water is maintained at either 4° or 27° C in a covered container providing protection from light. In use, stock Rivanol is diluted with carbonate buffer pH 9.6. Rivanol levels of 0.1 to 3.2% increase binding of proteins to polystyrene. Protein binding to polystyrene can be enhanced by rivanol utilizing two techniques: 1.) precoating of the polystyrene surface with Rivanol, followed by washing of the polystyrene and subsequent exposure of the polystyrene to protein; 2.) incubation of polystyrene with combined protein and Rivanol solution.

Technique 1. Stock Rivanol diluted in carbonate buffer is placed in polystyrene enzyme immunoassay (EIA) plate wells. Post incubation, the EIA plate wells are washed with phosphate buffer. Phosphate buffer containing the protein to be bound is then placed in the plate wells. The plate is incubated for an hour or more, and the wells are then washed with phosphate buffer followed by the addition of enzyme labelled antibody specific for the plated protein. After an hour of incubation the excess detecting antibody is washed away, and enzyme substrate is placed in the wells. Color development is stopped and absorbence is determined. The level of color produced is directly proportional to the amount of protein attached to the polystyrene surface of the well. With most proteins the level of color produced when Rivanol is associated with binding of the protein, either by pretreatment or by concomitant exposure of protein and Rivanol, as described below, is greater than that found when protein is plated in the absence of Rivanol.

Technique 2. Stock Rivanol diluted with carbonate buffer and containing the protein to be plated is placed in EIA plate wells. The concentration of Rivanol as well as temperature and period of incubation to produce optimal binding of the protein in question are determined empirically. Post incubation, the plate wells are washed with phosphate buffer, and antibody specific for the protein to be plated is placed in the wells. The remaining procedure is as described above in technique 1.

The protein in solution which is to be detected utilizing ELISA techniques with the incorporation of Rivanol (or other acridine dyes) may be derived from a pure protein preparation or from a complex solution such as serum.

Binding of protein to polystyrene occurs due to hydrophobic interactions. The ability of protein to bind to polystyrene varies with the protein in question. For example, acetylcholinesterase does not bind to polystyrene as well as does serum albumin. The limiting step in an EIA is the binding of protein to the solid phase. Rivanol is inexpensive, increases the binding of protein to polystyrene, increases the sensitivity of the ELISA and also allows a decrease in the time period required to perform an EIA.

The ability of Rivanol to increase the binding of proteins to polystyrene has been examined using horseradish peroxidase (HRP) labelled goat, sheep and rabbit immunoglobulin G (IgG). HRP labelled sheep, goat and rabbit IgG diluted in phosphate buffer at the levels indicated in the attached Figures (1,A&B;2,A&B;3,A&B) was incubated in EIA plate wells pre-coated with the rivanol concentrations and for the periods indicated. The period of incubation of the HRP labelled IgGs was one hour. Post incubation the wells were washed, and citrate buffer containing substrate for the HRP enzyme was placed in the wells. Color development was stopped after 15 min by the addition of 4N H_2SO_4. As indicated in the attached figures, 24 hours of pretreatment with 1.6% 15mM Rivanol results in optimal binding of all types of IgG.

The above data demonstrates the major problem in the utilization of Rivanol to increase binding of protein to polystyrene. After the desired protein is bound to the Rivanol activated polystyrene, subsequently added proteins also bind. Thus the identification of a protein bound to polystyrene in the presence of Rivanol with HRP labelled specific antibody results in the binding of the HRP labelled antibody. This action completely obscures any specificity by increasing the background color. To alleviate this problem the blocking agents gelatin and bovine serum albumin (BSA) have been tested for their ability to block the protein binding activity of Rivanol after the desired protein is bound. The results (Figures 4,5) suggest that the use of either BSA or gelatin at a level of 1% in phosphate buffer may block binding of subsequently added protein by Rivanol. The effectiveness of the blockade is dependent upon the substance used as a blocker and whether the blocking substance is added simultaneous with or prior to (sequential) exposure of the treated polystyrene plate to protein. Subsequent work suggests that the inclusion of Tween-20 in solutions containing protein decreases the binding of that protein to the Rivanol activated polystyrene surface. The source of the Tween-20 also appears to be of considerable importance. Tween-20 obtained from Bio-Rad (EIA grade) is superior to that obtained from Sigma Chemicals in

preventing attachment of protein to Rivanol activated polystyrene. This is adequately indicated through the following experiment (Figure 6). Microtiter plate wells were treated with the indicated levels of Rivanol in carbonate buffer for 16 hours at RT. Post washing tenth ml volumes of goat anti mouse IgG-HRP diluted 1:1000 in washing buffer containing 0.05% Tween-20 from either Bio-Rad or Sigma Chemicals was placed in the appropriate wells. The plate was incubated for one hour, washed and substrate for HRP placed in the wells. Color development was stopped after 15 min. From Figure 6 it is obvious that Bio-Rad Tween-20 decreases binding of the HRP labeled protein to the Rivanol activated polystyrene. Use of higher dilutions of the proteins utilized for detection of the protein primarily bound to Rivanol activated polystyrene also results in reduction of unwanted protein binding.

Utilizing antibody diluted in washing buffer containing 0.05% Tween-20 (Bio-Rad), human transferrin attached to Rivanol activated polystyrene was detected without interference from high background color levels due to attachment of subsequently added proteins. In brief, both the sequential and simultaneous methods of binding protein (in this case human transferrin) to polystyrene microtiter plate wells with Rivanol were utilized. (See above:Techniques 1&2). Time periods of 3 and 16 hours were employed. After binding of transferrin, goat anti-human transferrin diluted 1:8000 in washing buffer /.05% Tween-20 was placed in the microtiter plate wells and the plate incubated for 1 hour at RT. Post incubation the wells were washed and rabbit anti goat IgG-HRP diluted 1:3000 was placed in each well. After 1 hour of incubation at RT followed by washing, HRP substrate was added and color development stopped after 15 min. Figures 7-10 indicate that simultaneous binding is more efficient than sequential binding, regardless of the time period employed. In both binding techniques the amount of binding of proteins utilized to identify the transferrin bound by the Rivanol activated polystyrene is maintained at low levels due by the presence of Tween-20 (Bio-Rad).

Other acridines also increase binding of proteins to polystyrene. Quinicrine, acridine orange, 9-amino acridine, acriflavin and Rivanol increase the binding of eel acetylcholinesterase to polystyrene, albeit at different efficiencies, (data not shown).

Currently, we are continuing to examine the efficiency of Rivanol in binding proteins, both in pure form and in serum, to polystyrene.

Figure 1A

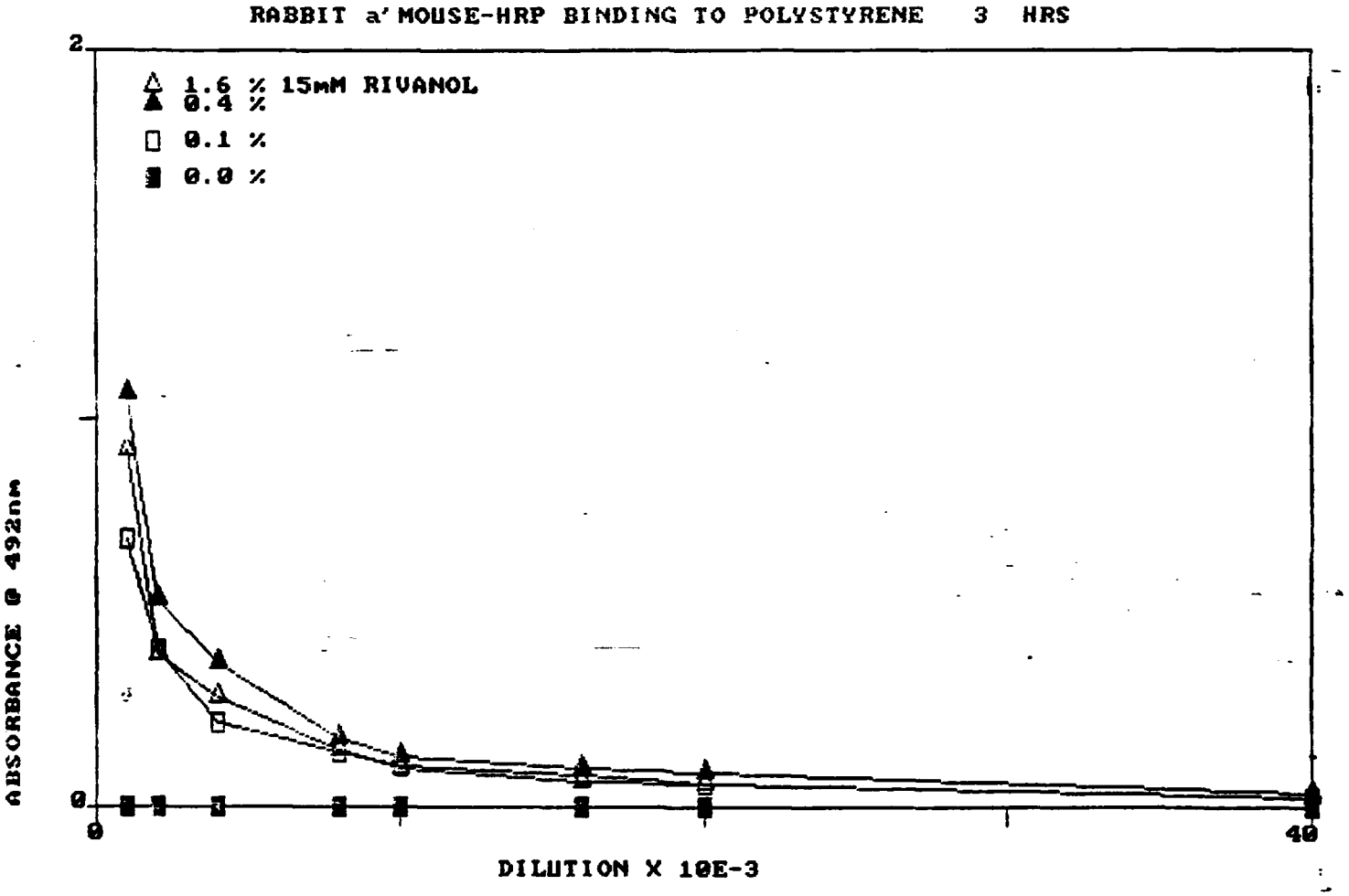

RABBIT a' MOUSE-HRP BINDING TO POLYSTYRENE 3 HRS
2
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
0
0
40
DILUTION X 10E-3

Figure 1B

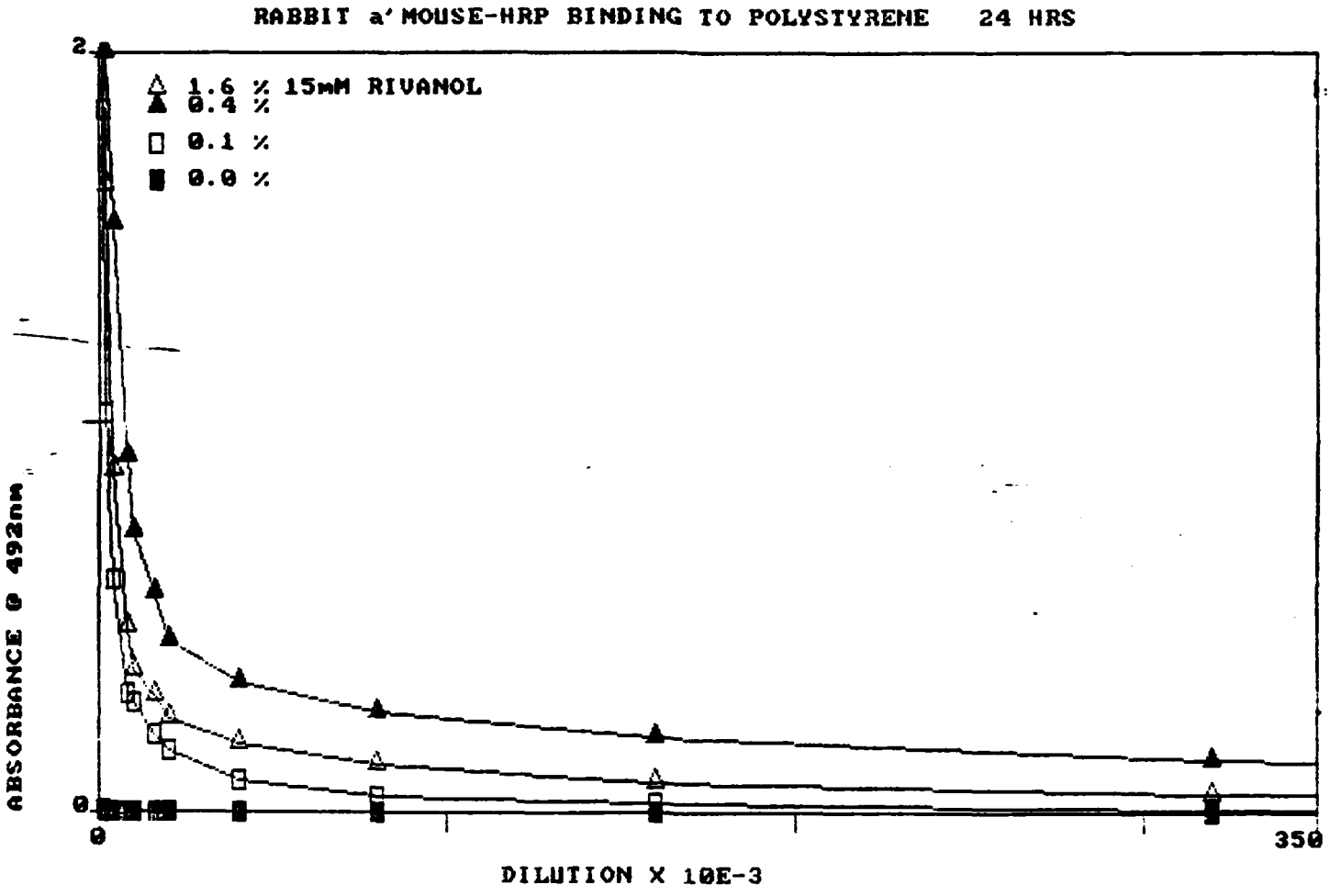

RABBIT a' MOUSE-HRP BINDING TO POLYSTYRENE 24 HRS
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
2
0
0
350
DILUTION X 10E-3

Figure 2A

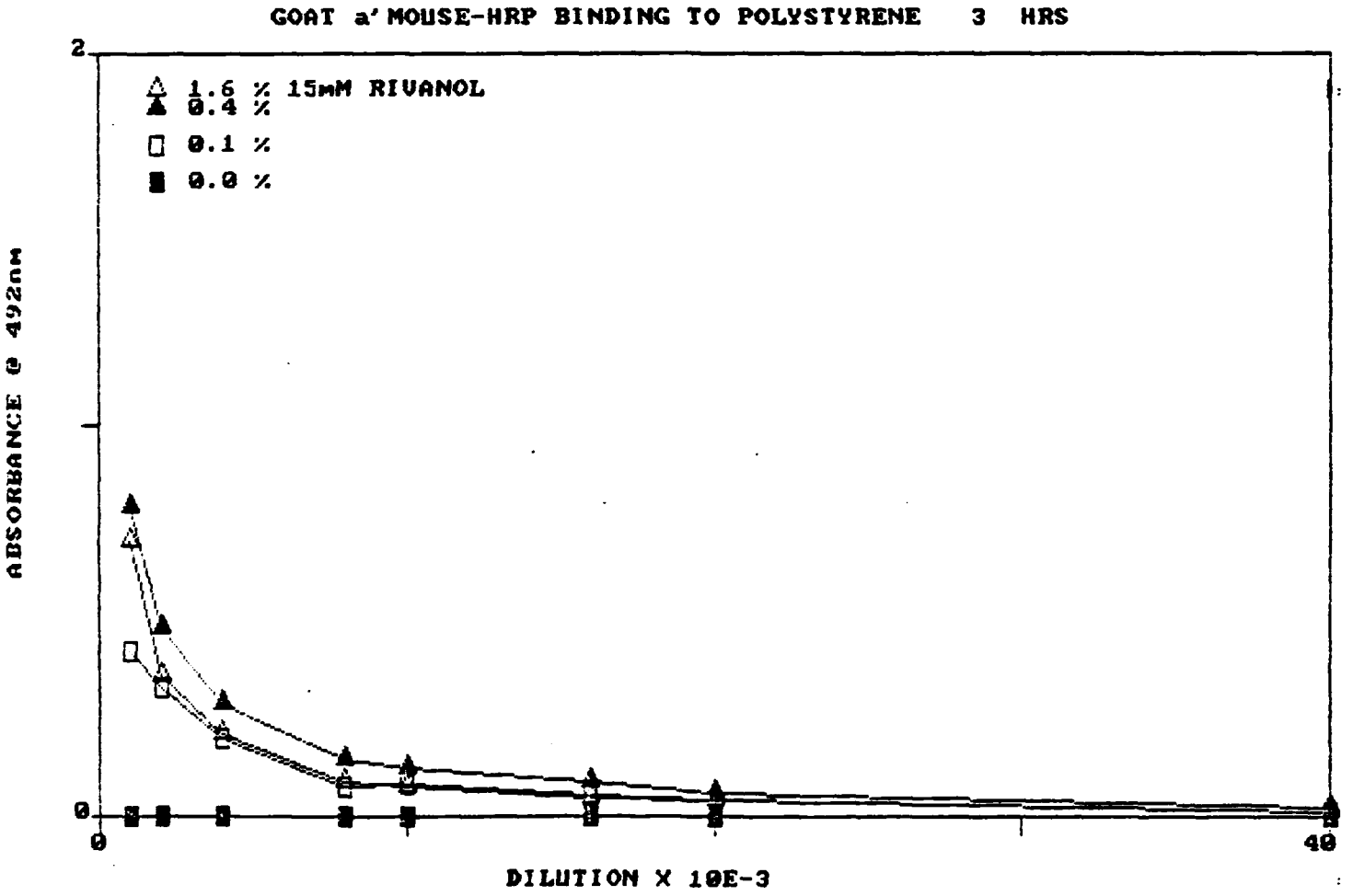

GOAT a' MOUSE-HRP BINDING TO POLYSTYRENE 3 HRS
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
2
0
0
40
DILUTION X 10E-3

Figure 2B

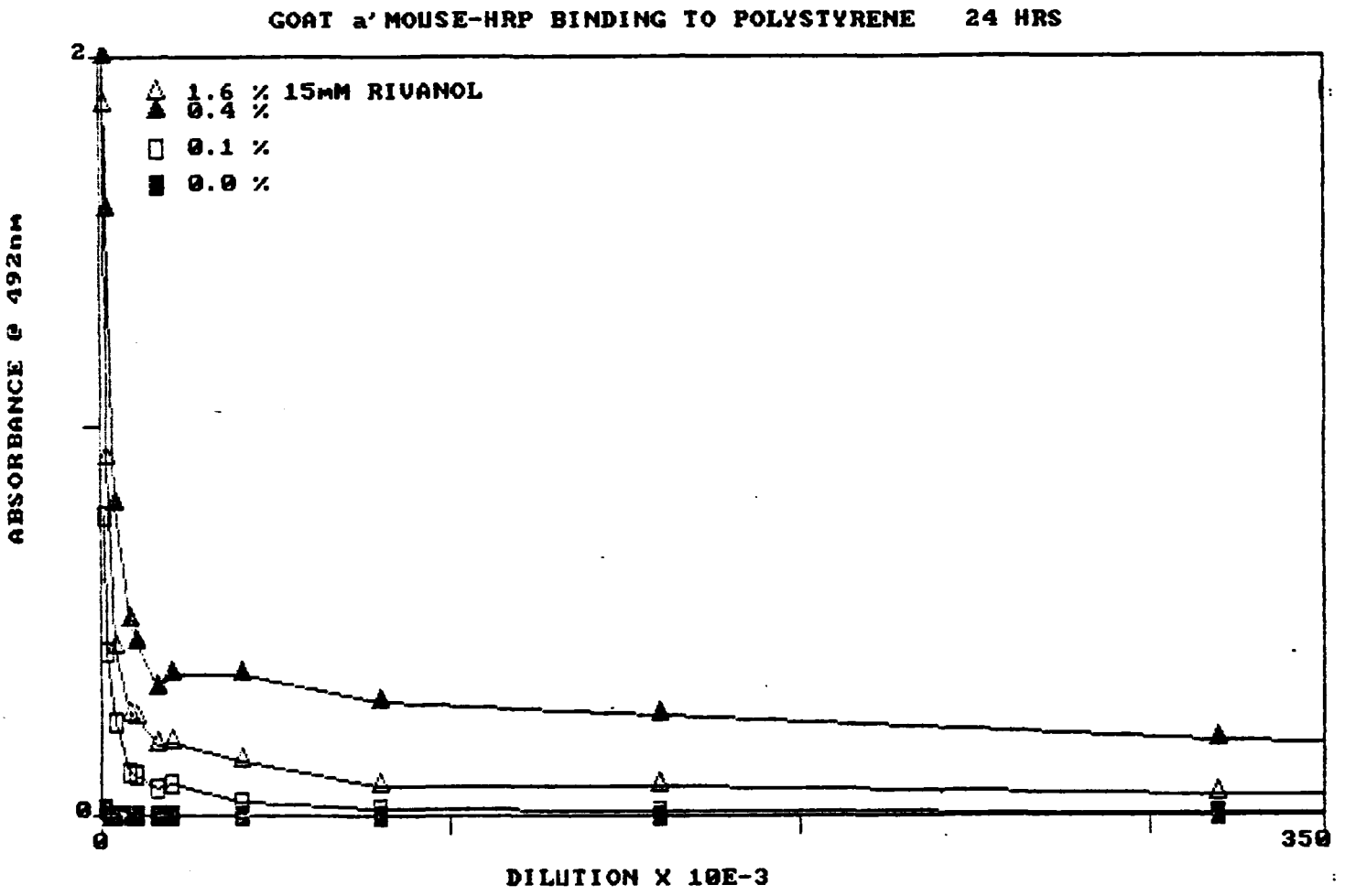

GOAT a' MOUSE-HRP BINDING TO POLYSTYRENE 24 HRS
2
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
0
0
350
DILUTION X 10E-3

Figure 3A

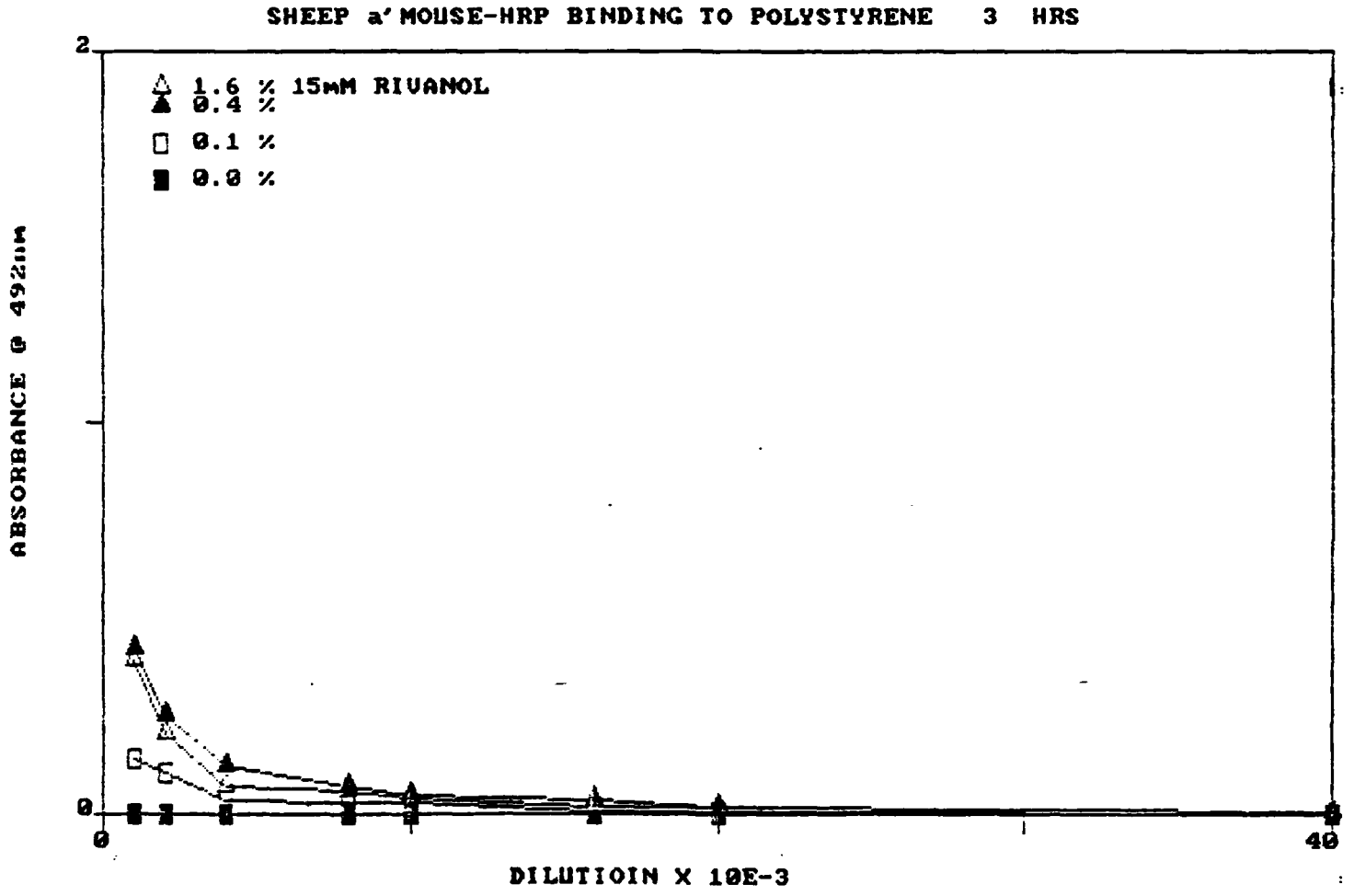

SHEEP a' MOUSE-HRP BINDING TO POLYSTYRENE 3 HRS
2
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
0
0
40
DILUTIOIN X 10E-3

Figure 3B

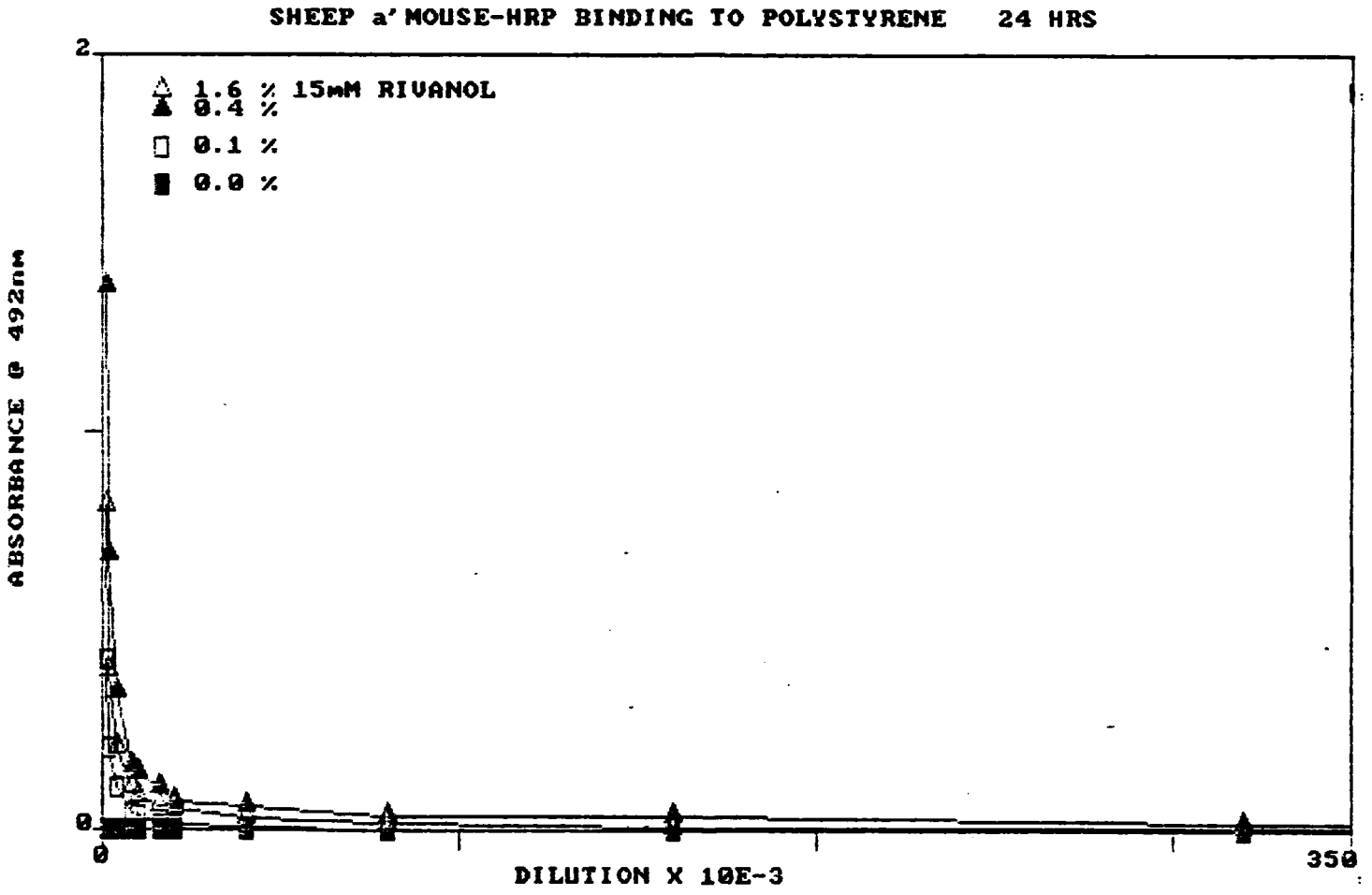

SHEEP a' MOUSE-HRP BINDING TO POLYSTYRENE 24 HRS
1.6 % 15mM RIVANOL
0.4 %
0.1 %
0.0 %
ABSORBANCE @ 492nm
2
0
0
350
DILUTION X 10E-3

Figure 4

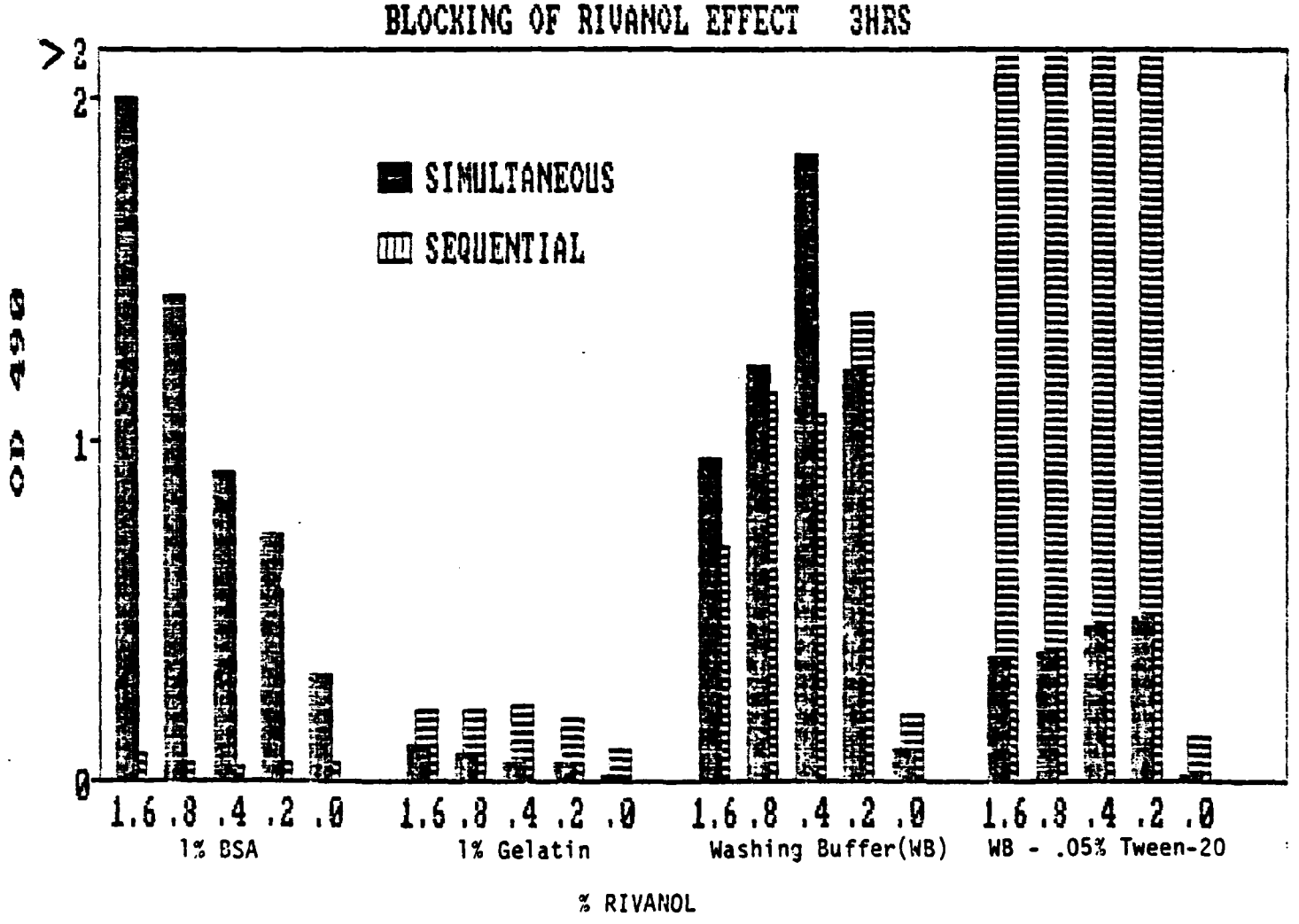

BLOCKING OF RIVANOL EFFECT 3HRS
>2
2
1
0
OD 490
SIMULTANEOUS
SEQUENTIAL
1.6 .8 .4 .2 .0
1% BSA
1% Gelatin
Washing Buffer(WB)
WB - .05% Tween-20
% RIVANOL

Figure 5

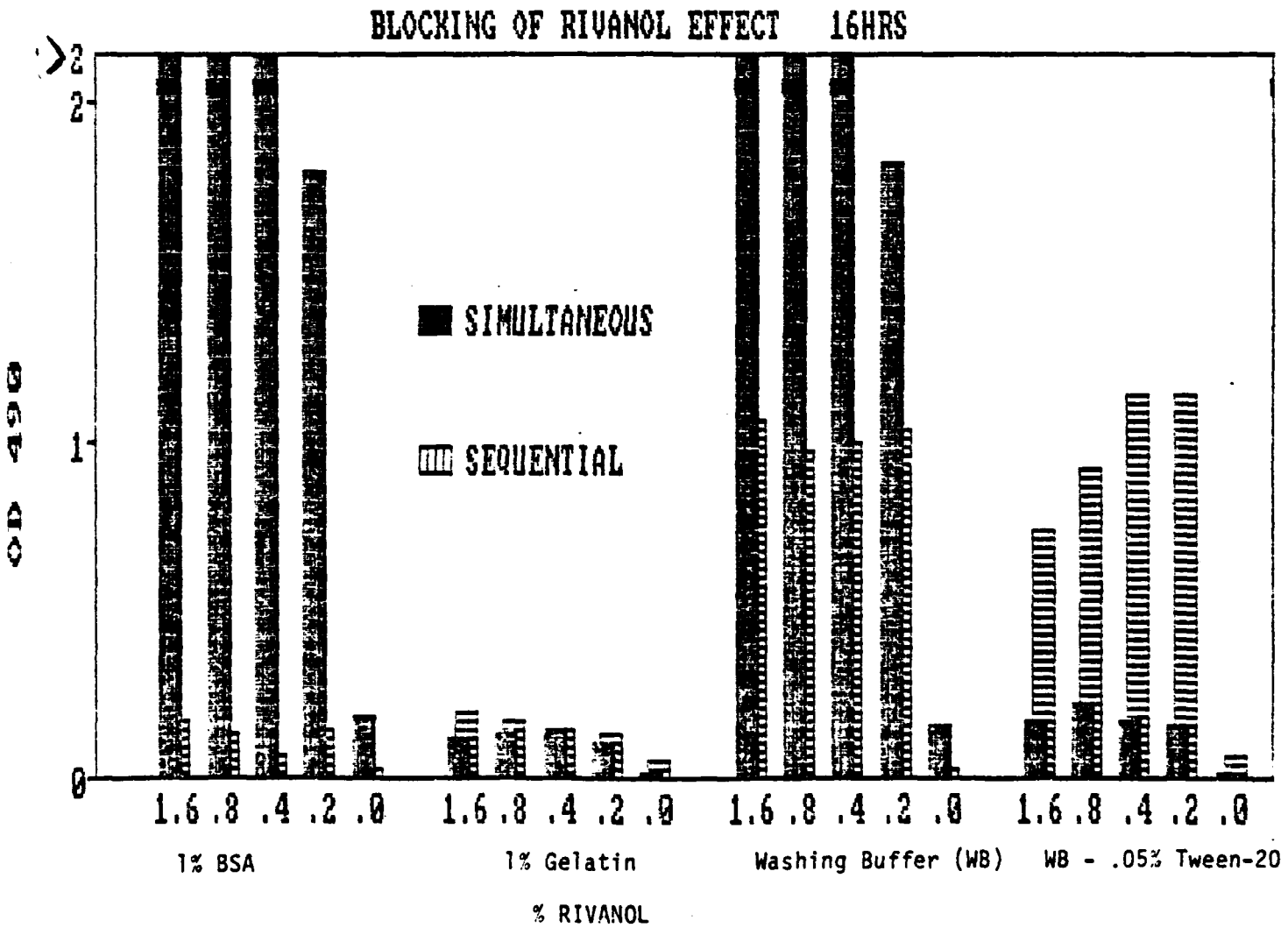

BLOCKING OF RIVANOL EFFECT 16HRS
>2
2
1
0
OD 490
SIMULTANEOUS
SEQUENTIAL
1.6 .8 .4 .2 .0
1.6 .8 .4 .2 .0
1.6 .8 .4 .2 .0
1.6 .8 .4 .2 .0
1% BSA
1% Gelatin
Washing Buffer (WB)
WB - .05% Tween-20
% RIVANOL

Figure 6

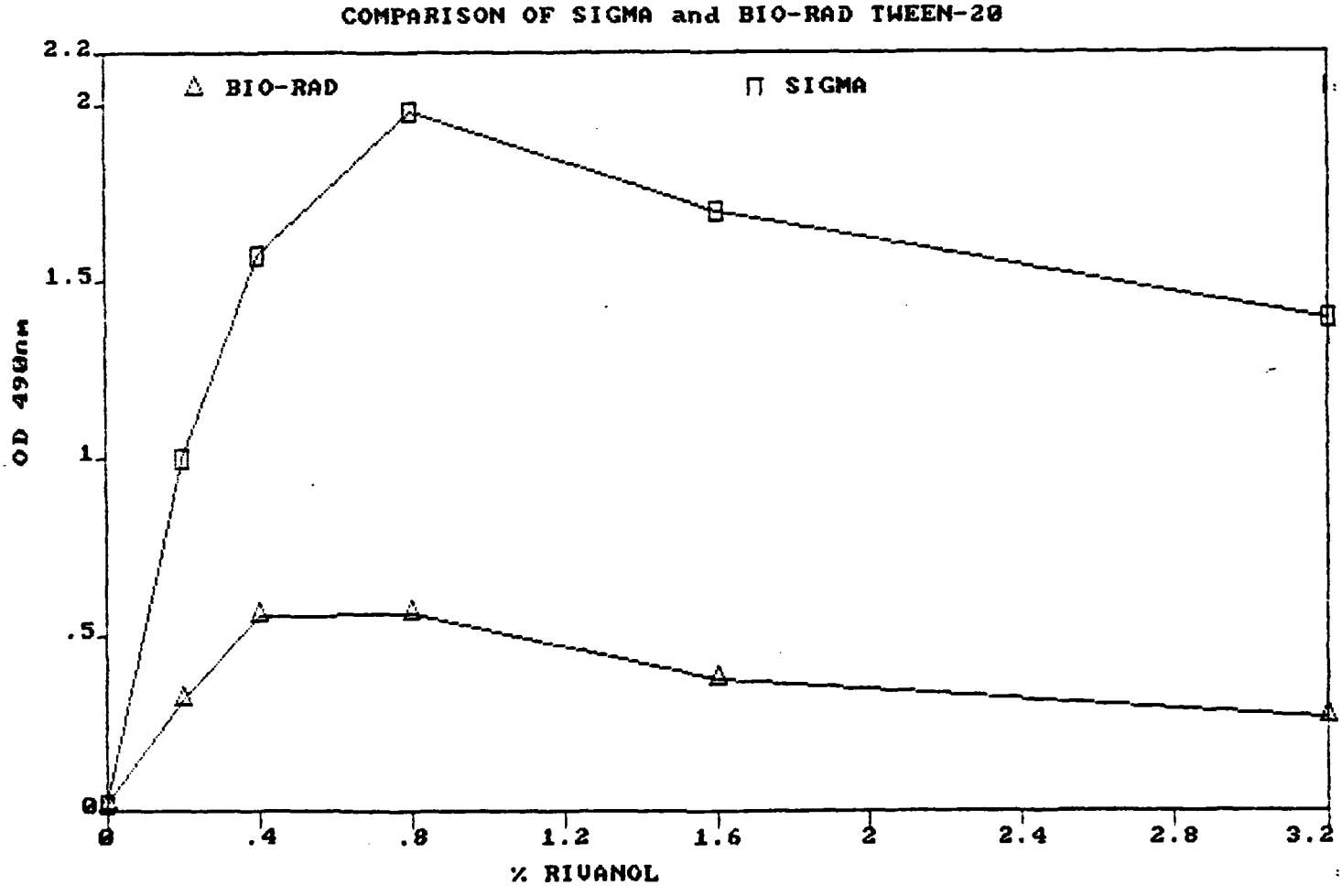

COMPARISON OF SIGMA and BIO-RAD TWEEN-20
△ BIO-RAD
□ SIGMA
OD 490nm
2.2
2
1.5
1
.5
0
0
.4
.8
1.2
1.6
2
2.4
2.8
3.2
% RIVANOL

Figure 7

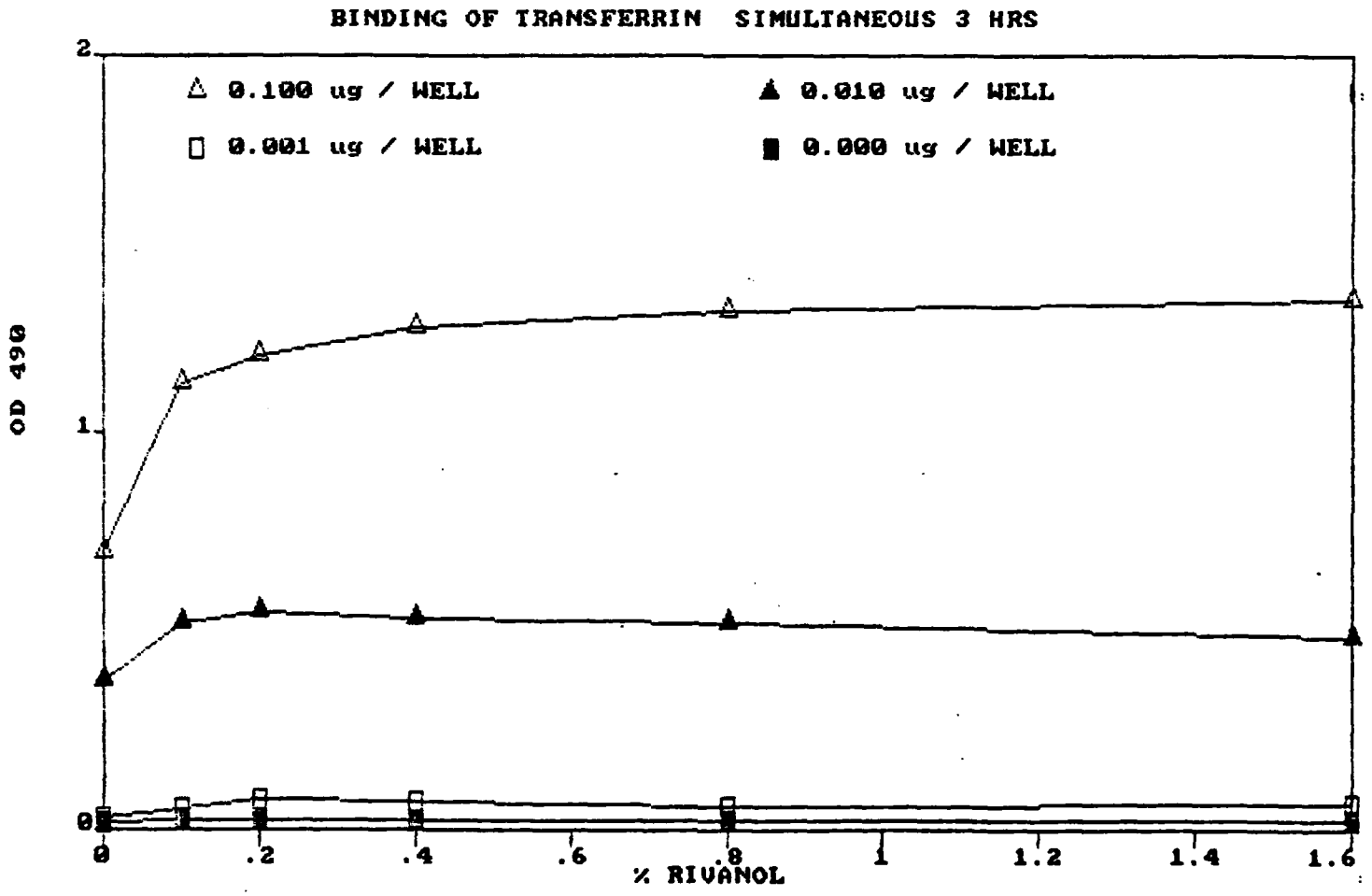

BINDING OF TRANSFERRIN SIMULTANEOUS 3 HRS
0.100 ug / WELL
0.010 ug / WELL
0.001 ug / WELL
0.000 ug / WELL
OD 490
2
1
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
% RIVANOL

Figure 8

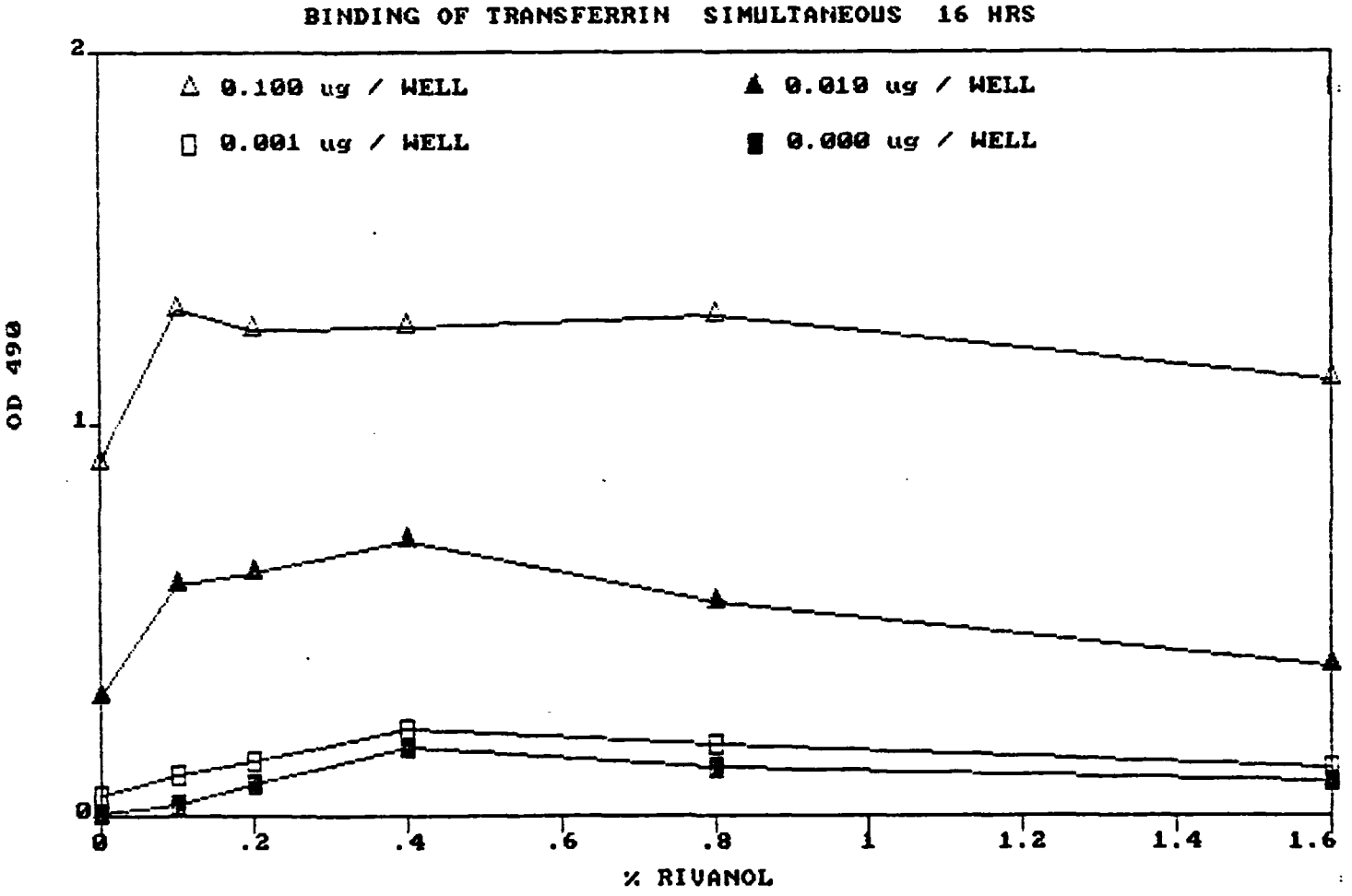

BINDING OF TRANSFERRIN SIMULTANEOUS 16 HRS
△ 0.100 ug / WELL
▲ 0.010 ug / WELL
□ 0.001 ug / WELL
■ 0.000 ug / WELL
OD 490
2
1
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
% RIVANOL

Figure 9

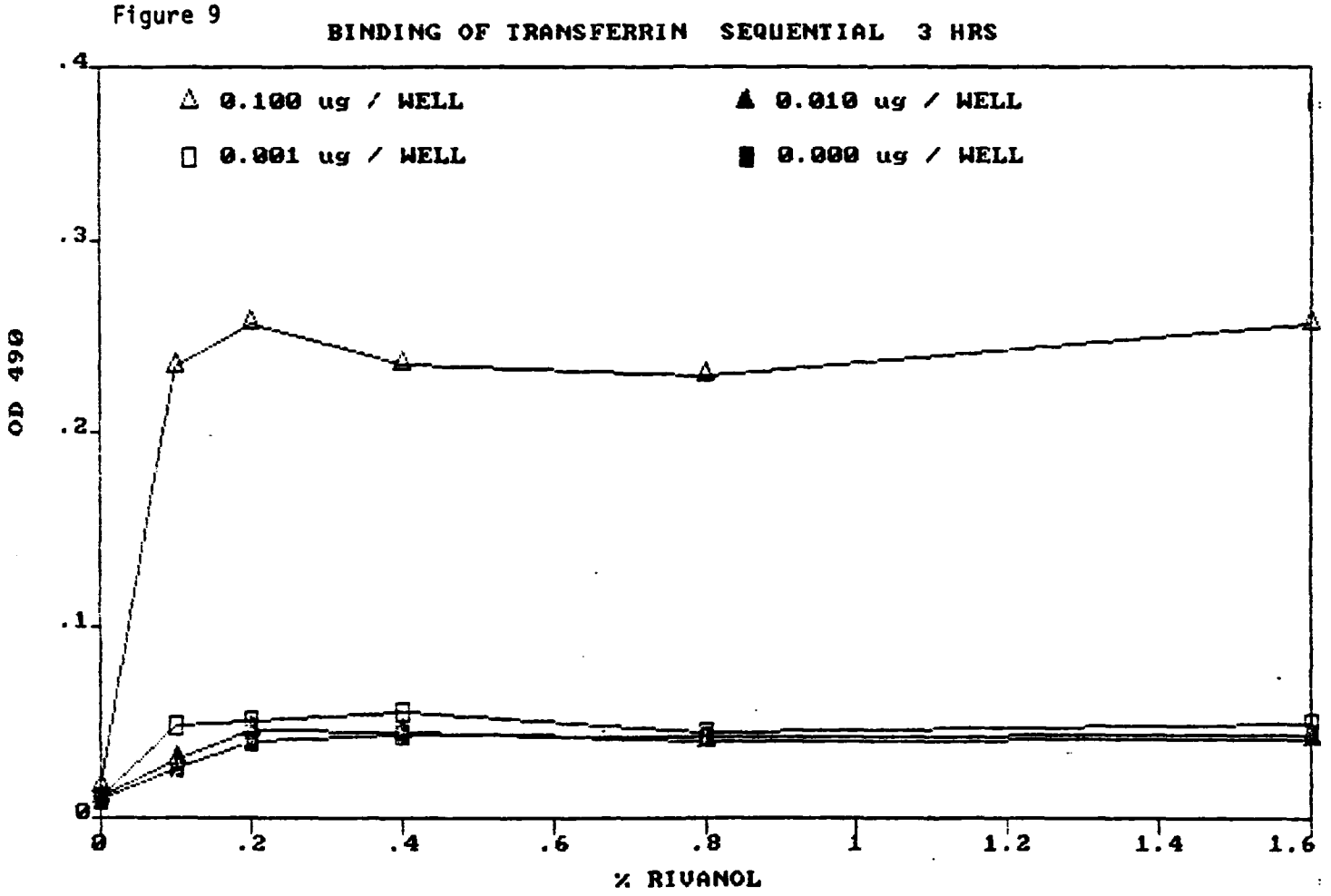
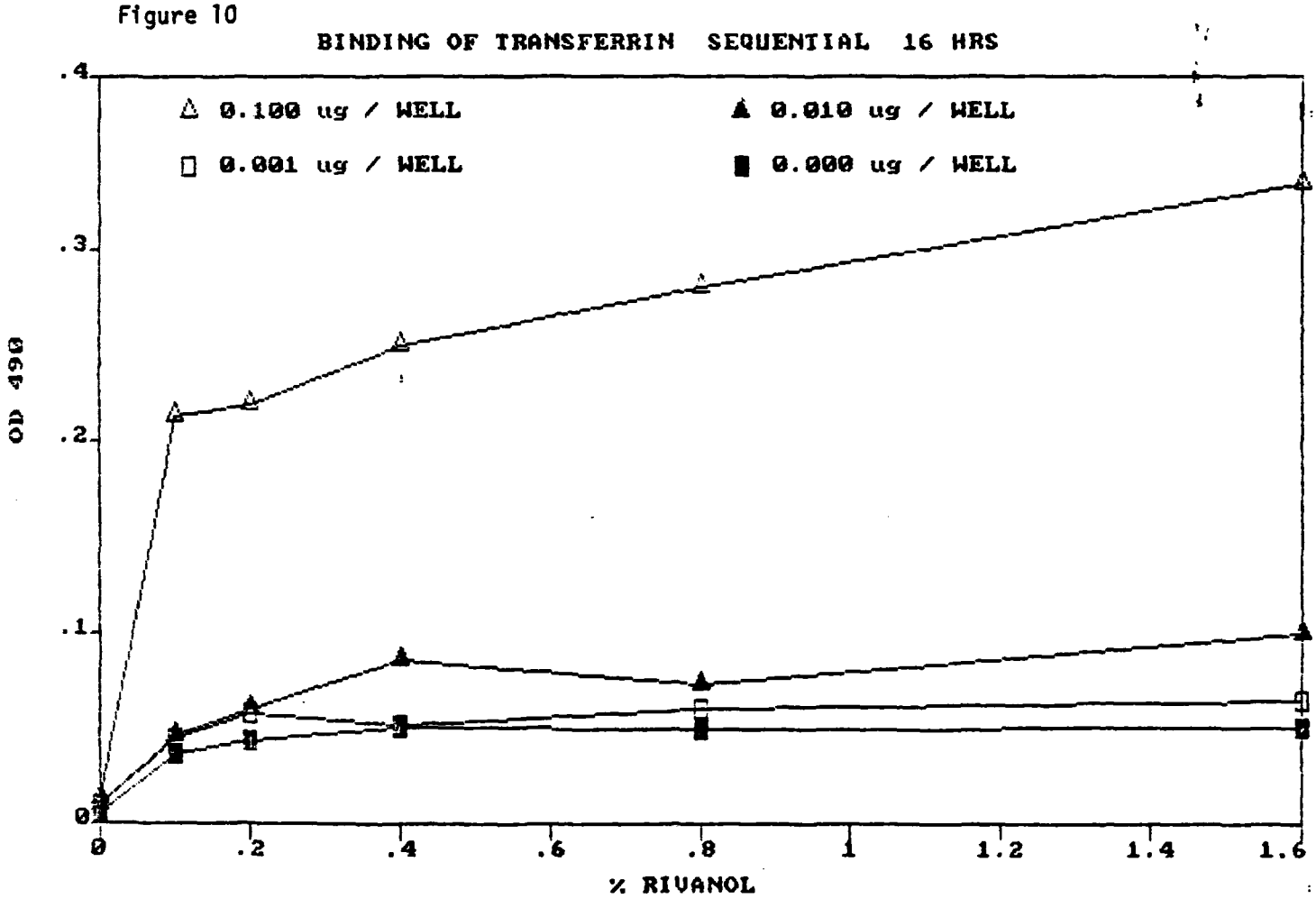

BINDING OF TRANSFERRIN SEQUENTIAL 3 HRS
0.100 ug / WELL
0.010 ug / WELL
0.001 ug / WELL
0.000 ug / WELL
OD 490
.4
.3
.2
.1
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
% RIVANOL

Figure 10


BINDING OF TRANSFERRIN SEQUENTIAL 16 HRS
0.100 ug / WELL
0.010 ug / WELL
0.001 ug / WELL
0.000 ug / WELL
OD 490
.4
.3
.2
.1
0
0
.2
.4
.6
.8
1
1.2
1.4
1.6
% RIVANOL

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